Cross-Reactivity of Murine Monoclonal Anti-DNA Antibodies with Human and Murine Skin: A Possible Pathogenetic Role in Skin Lesions of Lupus Erythematosus

Setsuya Aiba, M.D., Osamu Yoshie, M.D., Yasushi Tomita, M.D., and Hachiro Tagami, M.D.
Department of Dermatology (SA, YT, HT) and Department of Bacteriology (OY), Tohoku University School of Medicine, Seiryomachi, Sendai, Japan

Anti-DNA autoantibodies are known to cross-react with a wide variety of substances including cell-surface molecules. Thus, we examined cross-reactivities of 20 murine monoclonal anti-DNA antibodies with normal human and mouse skin tissues. Hybridomas producing these monoclonal antibodies were established from non-immunized spleen cells from autoimmune MRL-*1pr/1pr* mice, a strain characterized by spontaneous development of SLE-like disorders including skin changes. They were selected based on their reactivity to DNA in a typical enzyme-linked immunosorbent assay, in which nine monoclonal antibodies were reactive with both double-stranded DNA and single-stranded DNA, whereas nine monoclonals were reactive only with single-stranded DNA. Even though only seven of them were observed to stain nuclei, most of the monoclonal antibodies revealed strong and distinct cross-reactivities to various components of the skin tissues including the epidermal basement membrane, keratinocytes at different locations of the epidermis, melanocytes, Langerhans cells, Thy-1$^+$ dendritic cells in the case of murine skin, and mast cells. Our results suggest a possible role of so-called anti-DNA antibodies with high or low affinities to DNA in the pathogenesis of cutaneous lesions of lupus erythematosus. *J Invest Dermatol 93:739–745, 1989*

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a high serum level of autoantibodies directed against double-stranded DNA (dsDNA) [1]. The major pathologic changes in SLE are glomerulonephritis and vasculitis, in which immune complexes containing DNA have been implicated [2,3]. In contrast, in the skin lesions of LE, vacuolar degeneration of basal layer, and patchy perivascular inflammatory infiltrations in the dermis (mainly located in the vicinity of pilosebaceous structures) are more pathognomonic than vasculitis [4]. Immunofluorescence studies have been used to reveal deposits of immunoglobulins and complements at the dermo-epidermal junction in affected and sometimes in uninvolved skin in SLE [4]. Joost [5], however, could not detect DNA in the immune deposits of SLE skin lesions. Therefore, unlike those of the renal lesions, the pathologic cutaneous changes in SLE patients may not be induced by the immune complexes containing DNA.

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Reprint requests to: Hachiro Tagami, M.D., Department of Dermatology, Tohoku University School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai 980, Japan.

Abbreviations:
ANA: antinuclear antibody
dsDNA: double-stranded DNA
ssDNA: single-stranded DNA
BSA: bovine serum albumin
FACS: fluorescence activated cell sorter
FANA: fluorescent antinuclear antibody
FBS: fetal bovine serum
PBS: phosphate-buffered saline
SLE: systemic lupus erythematosus

It has been demonstrated that anti-DNA antibodies are cross-reactive with several different molecules such as cell-surface proteins known as lupus-associated membrane proteins (LAMP) [6], phospholipids [7], glycosaminoglycans such as heparan sulfate [8] and extractable nuclear antigens such as Sm RNP, SS-A, and SS-B antigens [9,10]. LAMP are present on the surface of many types of cells including lymphocytes, erythrocytes, platelets, glomerular cells, and neuronal cells, all known to be affected by SLE pathogenesis [6], whereas heparan sulfate is a major constituent of the glomerular and cutaneous basement membranes [11]. Recent studies of SS-A/Ro provide clues as to the role of antinuclear antibodies (ANA) in the pathogenesis of photosensitive cutaneous lupus erythematosus that shows keratinocyte damage following ultraviolet irradiation and estrogen treatment [12–14]. SS-A and SS-B antigens are expressed in an increased amount on the surface of keratinocytes to become a target of immune-mediated cell cytotoxicity in photosensitive cutaneous LE [12,13]. Thus, it is reasonable to speculate that the cross-reactive binding of anti-DNA antibodies to tissue antigens may also play a role in the production of various tissue injuries, including cutaneous lupus lesions.

In the present study, as a first step to elucidating the role of anti-DNA antibodies in the pathogenesis of SLE skin lesions, we examined the cross-reactivity of a large number of murine monoclonal anti-DNA antibodies with various components of normal human and mouse skin tissues. These monoclonal antibodies were derived from the spleen cells of MRL-*1pr/1pr* mice, a strain characterized by spontaneous development of SLE-like disorders including skin changes [15]. Our results revealed wide cross-reactivities of anti-DNA antibodies with various components of skin, suggesting a possible role of anti-DNA antibodies in the pathogenesis of cutaneous lesions of SLE.

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MATERIALS AND METHODS

DNA Both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) [16] were kindly provided by Dr. T. Sasaki of the Second Department of Internal Medicine, Tohoku University School of Medicine. In brief, calf thymus DNA was treated with RNase A, purified with phenol and chloroform-isoamyl alcohol extraction, and sonicated for 10 min. dsDNA was prepared from the sonicated DNA by means of digestion with nuclease S1. ssDNA was prepared from the sonicated DNA by means of thermal denaturation at 100°C, followed by rapid chilling with ice. Synthetic polynucleotides [poly (dA) poly(dT), poly(dG) poly(dC), poly(dA), poly(dT), poly(dG), and poly(dC)] were purchased from Pharmacia (Piscataway, NJ).

Isolation of Hybridomas Spleen cells obtained from MRL-*lpr/lpr* were fused with murine SP2 myeloma cells following the procedure described by Galfre et al [17]. The cells were cultured in 96-well type microtest plates under hypoxanthine-aminopterin-thymidine (HAT) selection. The culture medium was RPMI-1640 supplemented with 15% fetal bovine serum (FBS). After 2 weeks, hybridoma culture supernatants were screened for reactivity to dsDNA and ssDNA by using an enzyme-linked immunosorbent assay (ELISA) (see below). Positive hybridomas were cloned in soft agarose. The isotype of monoclonal antibodies was determined by using a Mouse Monoclonal Typing Kit (Serotec, Oxon, UK).

ELISA for Detection of Anti-DNA Antibodies ELISA for detection of anti-DNA antibodies was carried out essentially following the procedure described by Faaber et al [8]. In brief, 96-well-type polystyrene microtest plates (Terumo, Tokyo, Japan) were precoated with protamine sulfate (Takeda, Osaka, Japan) (0.5 mg/ml, 150 μl/well) at room temperature for 2 h. After washing with phosphate-buffered saline (PBS), the plates were coated with ssDNA or dsDNA (50 μg/ml in PBS, 100 μl/well) at 4°C overnight. The antigen concentration used for coating was in excess of the optimal concentration. In some experiments, synthetic polydeoxynucleotides were employed for coating. After washing with PBS containing 0.05% Tween 20, the plates were blocked with PBS containing 5% bovine serum albumin (BSA) (Sigma) (200 μl/well) at room temperature for 2 h. Hybridoma supernatants (original or serially diluted with RPMI-1640 supplemented with 15% FBS) were added to the wells (50 μ/well) and incubated at room temperature for 2 h. After washing with 0.05% Tween 20-PBS, biotin-labeled horse anti-mouse IgG (Vector Laboratories., Inc., Burlingame, CA) was added to each well (1.5 μg/ml in 5% BSA-PBS, 100 μl/well), and incubation was carried out at room temperature for 1 h. After washing with 0.05% Tween 20-PBS, streptavidin-biotinylated horseradish peroxidase complex (Amersham International plc., Buckinghamshire, England) (1:1,000 in 5% BSA-PBS, 100 μl/well) was added to each well and incubated at room temperature for 1 h. After washing with 0.05% Tween 20-PBS, peroxidase substrate solution (ABTS, KPL Inc., Gaithersburg, MD) was added to each well (100 μl/well). After incubation at room temperature for 30 min, an optical density at 405 nm was read with a two-wavelength microplate photometer (MTP-12, Corona Electric, Ibaraki, Japan). Blank wells were made by incubation with RPMI-1640 supplemented with 15% FBS (instead of with hybridoma culture supernatants), and $OD_{405} > 1.0$ was taken to be positive. Murine monoclonal antibodies of various isotypes with unrelated specificities (Y14: IgM anti-HLA, Y185: IgG, anti-HLA, S5-1: IgG**2a** anti-sheep erythrocytes, NS-8: IgG**2b** anti-sheep erythrocytes) were also used as isotype controls in ELISA and constantly showed no reactivity above the blank level.

Indirect Immunofluorescence with Monoclonal Antibodies Normal human skin obtained from five individuals and normal skin from nine BALB/c mice were used as substrates. Cryostat sections (6 μm thick) prepared from the frozen human skin and BALB/c mouse skin were allowed to air-dry at room temperature. The sections were overlaid with culture supernatants of anti-DNA hybridomas or isotype controls and incubated in a moist chamber at 4°C overnight. After washing with PBS, the sections were incubated with FITC-conjugated goat anti-mouse immunoglobulins (Tago Inc., Burlingame, CA) (1:40 dilution in PBS) at 37°C for 1 h. After washing with PBS, the sections were mounted in Perma Fluor (Lipshaw, Detroit, MI) and observed under an Olympus Vanox fluorescent microscope.

For double staining with anti-tyrosinase and anti-DNA antibodies, sections were first incubated with a combination of rat monoclonal antibody against tyrosinase [18] and one of murine monoclonal anti-DNA antibodies, and then were stained with a combination of FITC-labeled anti-rat immunoglobulins with minimum cross-reactivity with mouse serum and rhodamine-conjugated $F(ab')_2$ anti-mouse immunoglobulins (Tago). For double staining with anti-DNA and anti-HLA-DR antibodies, cryostat sections were sequentially incubated with those of murine monoclonal anti-DNA antibodies, then with FITC-labeled $F(ab')_2$ anti-mouse immunoglobulins, with 50% mouse serum in PBS, and finally with phycoerythrin-conjugated anti-HLA-DR antibody (Becton Dickinson, Mountain View, CA). The mounted sections were observed with an appropriate filter setting for FITC, rhodamine, or phycoerythrin.

Immunofluorescence Staining of Murine Epidermal Sheets Immunofluorescence staining of epidermal sheets was performed by the method described elsewhere [19]. Briefly, the amputated ears of mice were cut into small 4–6-mm square pieces and treated with 20 mM EDTA in PBS at 37°C for 2 h. The epidermal sheets thus obtained were washed in PBS, fixed in absolute ethanol, rehydrated in PBS, and incubated in 2% BSA in PBS. Then they were incubated with mouse monoclonal anti-DNA antibody 12-C-8 and rat anti-mouse Thy-1.2 antibody (Becton Dickinson) at 4°C overnight. After being washed three times in PBS, they were further incubated with rhodamine-labeled anti-mouse immunoglobulins (Tago) and FITC-conjugated anti-rat immunoglobulins (Tago). After washing with PBS, the epidermal sheets were mounted and observed with Olympus fluorescence microscopy.

Fluorescent Antinuclear Antibody (FANA) Test Undiluted samples were screened for ANA by a standard indirect immunofluorescence technique using cultured HeLa S3 cells as a substrate.

Immunoperoxidase and Toluidine Blue Double Staining Cryostat sections (6 μm thick) of human skin and BALB/c mouse skin were air-dried and fixed in acetone for 30 min. The sections were then blocked with 1% horse serum in PBS and incubated with one of the monoclonal anti-DNA antibodies or isotype controls at 4°C overnight. After being washed with PBS, the sections were treated with biotin-conjugated horse anti-mouse IgG and preformed avidin-biotin-peroxidase complex (Vector Lab. Inc., Burlingame, CA) at 37°C. After washing again with PBS, the sections were incubated in 0.05M Tris-HCl buffer (pH 7.6) containing 0.05% 3,3'-diamino-benzidine-tetrahydrochloride, and 0.01% hydrogen peroxide for 5 min at room temperature. After another washing, the sections were stained with toluidine blue, processed from distilled water to xylene, and mounted in mounting agent (Eukit).

RESULTS

Isolation of Murine Anti-DNA Monoclonal Antibodies Hybridomas were produced from the spleen cells of autoimmune MRL-*lpr/lpr* mice. Two fusion experiments were carried out, the first one using a 6-month-old mouse as a spleen cell source and the second using a 3-month-old mouse. Each fusion produced about 1,000 hybridomas, and those producing anti-DNA antibodies were screened with ELISA, using microtest plates coated with either dsDNA or ssDNA. Positive hybridomas were subsequently cloned. Clones obtained from the first fusion numbered from 1 to 10 in the first part of their designation, whereas those obtained from the second fusion numbered from 11 to 20. As shown in Table I, eleven monoclonal antibodies reacted with both dsDNA and ssDNA (Group A), whereas nine monoclonal antibodies reacted mainly with ssDNA (Group B). Monoclonal antibodies reacting only with

Table I. Class, Reactivities Against dsDNA and ssDNA in ELISA, and ANA Activity of Monoclonal Antibodies

Monoclonal Antibodies	Class	Reactivity ssDNA	Reactivity dsDNA	FANA[a]
Group A				
1-C-1	IgM	3^7	3^2	−
1-C-8	IgM	3^7	3^2	−
1-G-6	IgG2a	3^4	3^3	+
2-E-11	IgG2b	3	3	+
3-H-12	IgG2a	3^2	3	+
7-C-6	IgG2b	3^3	3^4	+
7-E-6	IgM	3^7	3	−
14-C-8	IgM	3^6	3^2	−
16-D-6	n.d.[b]	3^3	3	−
16-G-2	IgGl	3^5	3^6	+
20-A-8	IgM	3^4	3^2	−
Group B				
3-A-8	IgM	3	<3	−
3-B-4	IgM	3^4	<3	−
4-E-12	IgM	3^6	<3	−
8-D-5	IgM	3^5	<3	−
10-A-8	IgM	3^4	<3	−
12-C-8	IgM	3^3	<3	−
13-C-11	IgM	3	<3	−
15-B-12	IgM	3^6	<3	−
16-H-1	IgM	3^6	<3	−

[a] HeLa S_3 cell used as a substrate for FANA.
[b] Could not be determined by the Serotec kit.

dsDNA were not found. Only five monoclonal antibodies in group A produced positive nuclear staining in FANA test using acetone-fixed Hela cells. They were all of the IgG class. This may be due to the well-known fact that IgG antibody generally has a higher specificity and affinity than IgM antibody.

We further examined the epitope specificity of each anti-DNA monoclonal antibody by ELISA using synthetic polynucleotides as coating antigens. Each of the group A monoclonal antibodies (Table II) showed little base specificity and bound to both poly (dA.dT) and poly (dC.dG) with a closely similar reactivity, indicating that they were directed to epitopes present in the framework structure of dsDNA. On the other hand, the group B monoclonal antibodies demonstrated variable base specificities (Table III). Four monoclonal antibodies (4-E-12, 8-D-5, 12-C-8, 16-H-1) reacted only with poly (dT); one (15-B-12) with poly (dT) and poly (dC); one (10-A-8) with poly (dA), poly (dG), and poly (dC); and one (10-A-8) with poly (dA), poly (dG), and poly (dC). One (3-A-8) bound to all four polynucleotides, while another (3-B-4) did not have any reactivity with any of the four homopolymers.

Immunofluorescence Staining of Human and Mouse Skin Tissues We first examined the reactivity of anti-DNA monoclonal antibodies with normal human skin by means of indirect immunofluorescence staining. The results of the 11 monoclonal antibodies reactive with both dsDNA and ssDNA (Table I, Group A) were as follows: six (1-G-6, 2-E-11, 3-H-12, 7-C-6, 16-D-6, 16-G-2) stained all nuclei (Fig 1*A*); two (7-E-6, 14-C-8) stained cytoplasms of only epidermal cells (Fig 1*B*); one (1-C-1) stained the cytoplasms of epidermal cells and those of some dermal cells (not shown); one (20-A-8) stained the cytoplasms of suprabasal keratinocytes, as well as some dendritic cells and dermal cells (Fig 1*C*); and one (1-C-8) showed no reactivity with human skin. The results concerning the nine monoclonal antibodies reactive with only ssDNA (Table I, Group B) were as follows: one (3-B-4) stained nuclei (Fig 1*D*); two (4-E-12, 15-B-12) stained the cytoplasms of epidermal cells (not shown); one (12-C-8) stained epidermal basal cells, epidermal dendritic cells, and dermal cells (Fig 1*E*); one (16-H-1) stained suprabasal keratinocytes as well as some dendritic cells and dermal cells (not shown); one (8-D-5) stained some dermal cells (Fig 1*F*); and three (3-A-8, 10-A-8, 13-C-11) showed no reactivity with human skin. These results are summarized in Table IV.

Table II. Reactivity of Group A MoAb to Synthetic Double-Stranded Polynucleotides

Group A MoAb	ELISA antigen poly(dA.dT)	ELISA antigen poly(dC.dG)
1-C-1	2^7	2^7
1-C-8	2^6	2^6
1-G-6	2^9	2^9
2-E-11	2^4	2^4
3-H-12	2^4	2^4
7-C-6	2^5	2^4
7-E-6	2^4	2^5
14-C-8	2^9	2^8
16-D-6	2^2	2^2
16-G-2	2^{10}	2^9
20-A-8	2^8	2^8

Table III. Reactivity of Group B MoAb to Synthetic Single-Stranded Polynucleotides

Group B MoAb	ELISA antigen poly(dA)	ELISA antigen poly(dG)	ELISA antigen poly(dT)	ELISA antigen poly(dC)
3-A-8	2^7	2^8	2^9	2^{10}
3-B-4	<2	<2	<2	<2
4-E-12	<2	<2	2^4	<2
8-D-5	<2	<2	2^7	<2
10-A-8	2^5	2^6	<2	2^6
12-C-8	<2	<2	2^9	<2
13-C-11	<2	<2	<2	2^2
15-B-12	<2	<2	2^4	2^2
16-H-1	<2	<2	2^9	<2

The staining pattern of the seven anti-nuclear antibodies (1-G-6, 2-E-11, 3-B-4, 3-H-12, 7-C-6, 16-D-6, 16-G-2) showed some differences. 1-G-6, 2-E-11, and 7-C-6 stained nuclei rather diffusely (Fig 2*A*). 7-C-6 also reacted with the epidermal basement membrane zone (Fig 2*A*). 3-H-12, 16-D-6, and 16-G-2 showed a granular pattern of nuclear staining. 3-H-12 and 16-G-2 stained many fine granules of nuclei (Fig 2*B*), while 16-D-6 stained more sparse and large granules (Fig 2*C*). 3-B-4 stained the nuclear membrane of epidermal and dermal cells (Fig. 1*D*).

We also examined the reactivity of anti-DNA monoclonal antibodies with the components of normal mouse skin. Interestingly, the results of indirect immunofluorescence staining of normal mouse skin tissue in 18 out of the 20 anti-DNA monoclonal antibodies were almost the same as those in human skin described above (Fig 3*A*, *B*). Two exceptions were 14-C-8, which stained epidermal cells in the human skin but did not react with those of the mouse skin, and 16-D-6, which stained nuclei in the human skin and reacted with some dermal cells in the mouse skin, possibly mast cells. The results are also summarized in Table IV.

Identification of Melanocytes, Langerhans Cells, Thy-1$^+$ Dendritic Epidermal Cells, and Mast Cells In order to determine the identity of dendritic cells in the basal layer recognized by 20-A-8, 12-C-8, or 16-H-1 (Table IV), double immunofluorescence stainings with one of these anti-DNA monoclonal antibodies and rat monoclonal anti-tyrosinase antibody were carried out. As shown in Fig 4*A*,*B*, the dendritic cells detected by these three anti-DNA monoclonal antibodies were tyrosinase-bearing melanocytes. Similarly, immunofluorescence stainings with monoclonal anti-DNA antibodies and anti-HLA-DR antibody showed that some dendritic cells detected by 12-C-8 in the suprabasal layer expressed HLA-DR antigen, suggesting that 12-C-8 was reactive with Langerhans cells (Fig 5*A*,*B*).

In observations using murine epidermal sheets, 12-C-8 reacted with Thy-1$^+$ dendritic epidermal cells other than Langerhans cells (Fig 6*A*,*B*).

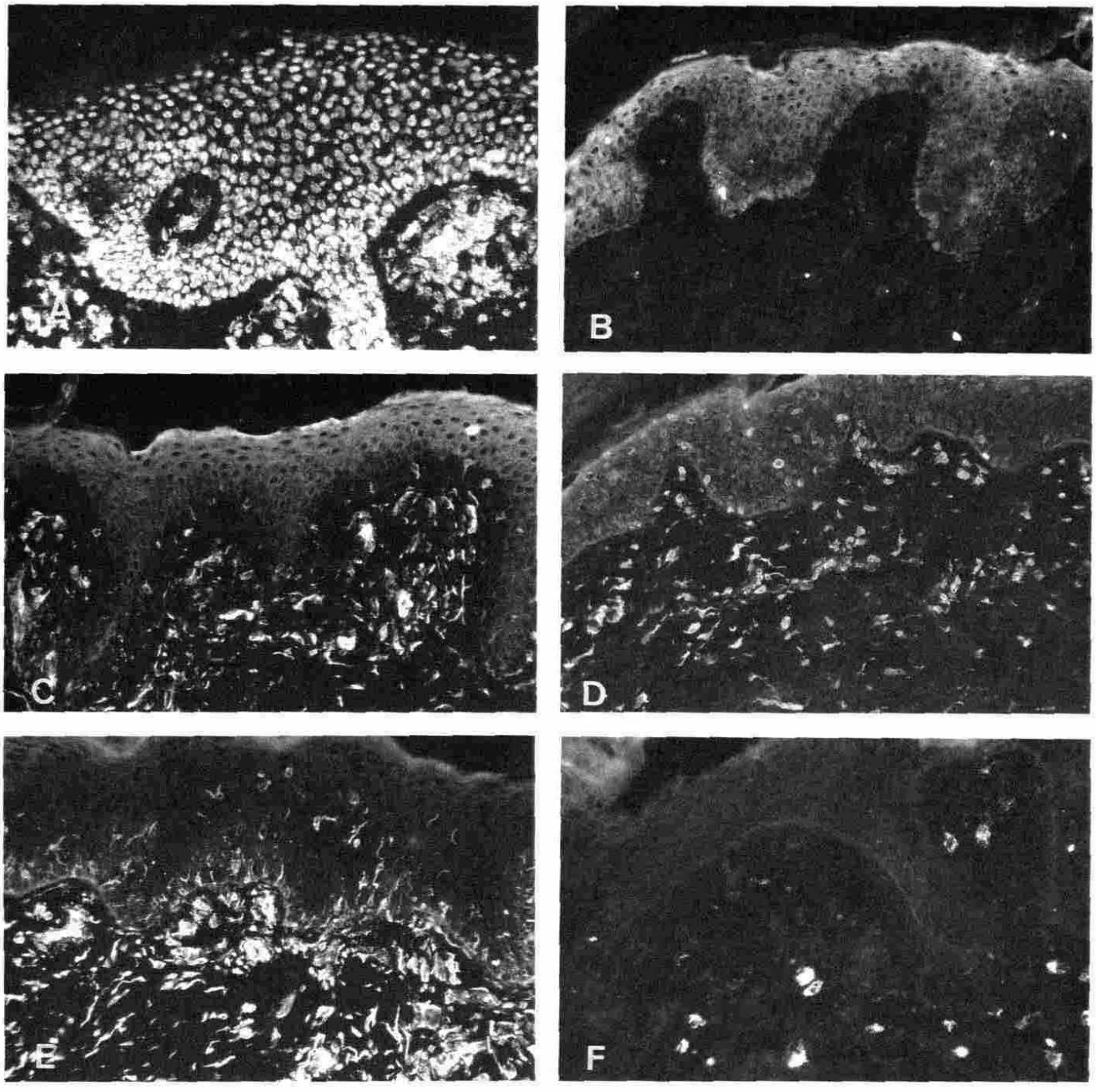

Figure 1. Immunofluorescence staining of human skin tissues with anti-DNA antibodies. 16-G-2 stained nuclei (*A*), 7-E-6 stained cytoplasm of epidermal cells (*B*), 16-H-1 stained the upper layer of epidermal cells with some epidermal dendritic cells and dermal cells (*C*), 3-B-4 stained nuclear membranes (*D*), 12-C-8 stained epidermal basal cells, epidermal dendritic cells, and dermal cells (*E*), and 8-D-5 stained some dermal cells (*F*) (× 150).

Because tissue mast cells in the skin can be detected with toluidine blue staining, double stainings with anti-DNA monoclonal antibodies and toluidine blue was carried out. As a result, 8-D-5 was found to be reactive to cutaneous mast cells (data not shown).

DISCUSSION

Production of anti-DNA antibodies is one of the most characteristic immunologic phenomena in SLE and in some other autoimmune diseases [1]. However, the mechanism for the production of anti-DNA antibodies or their role in the pathogenesis of SLE, particularly of its skin lesions, is still largely unknown. Recent studies demonstrating that anti-DNA antibodies are cross-reactive with a wide variety of molecules, such as cell-surface antigens (LAMP) [9], phospholipids [10], glycosaminoglycans [11], and extractable nuclear antigens [12,13], may provide important clues to solving these unanswered questions. Thus, in the present study, we examined the reactivity of 20 murine monoclonal antibodies that were reactive to DNA in ELISA with components of normal human and mouse skin. The monoclonal antibodies were derived from hybridomas that were produced from the spleen cells of MRL-*1pr/1pr* mice, a strain characterized by spontaneous development of SLE-like disorders [15]. In ELISA, 11 monoclonals bound to both dsDNA and ssDNA (Group A in Table I), whereas the other nine monoclonals bound only to ssDNA (Group B in Table I).

Our findings, however, indicate that the anti-DNA antibodies selected by anti-DNA ELISA contained those with low affinities to

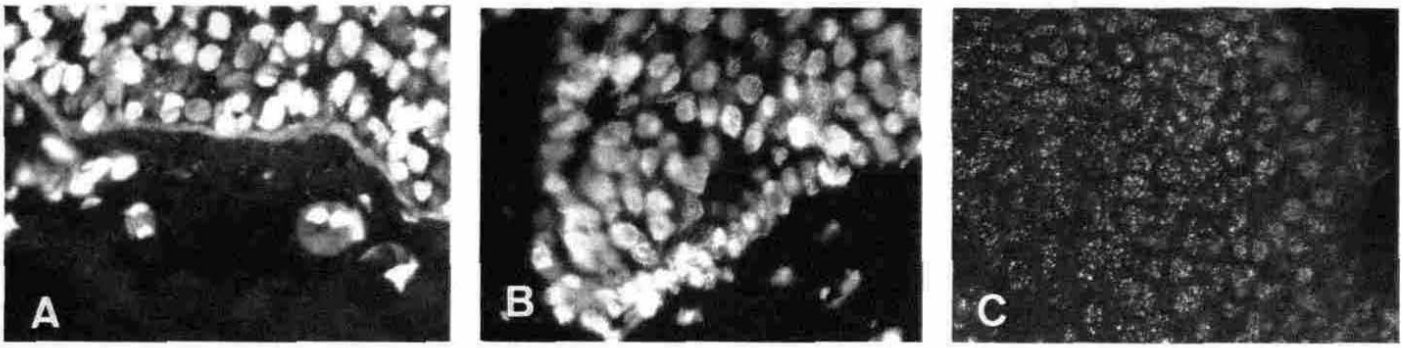

Figure 2. Indirect immunofluorescence staining of anti-nuclear antibodies. Diffuse staining with 7-C-6 (*A*), many granules stained with 16-G-2 (*B*), and sparse granules stained with 16-D-6 (*C*). 7-C-6 also reacted with the epidermal basement membrane zone (× 300).

DNA because only some of them showed FANA positivity or nuclear staining in fresh frozen tissues (Tables I and IV). Recently Smeenk et al [20] also reported that anti-DNA ELISA was selective for monoclonals with lower affinities among those derived from hybridomas prepared from MRL-*1pr/1pr*, NZB/W, and graft-vs-host-diseased mice. Furthermore, they found that there was an inverse relationship between anti-DNA avidity and cross-reactivity to other antigens. Our present results also clearly demonstrated wide cross-reactivities of such low affinity anti-DNA antibodies to normal tissue components (see below).

Our indirect immunofluorescence staining revealed that 16 out of the 20 anti-DNA monoclonal antibodies reacted with at least one of the components in normal human skin. Seven monoclonals were found to be anti-nuclear antibodies, while five were observed to stain the cytoplasms of epidermal cells or dermal cells. Three other monoclonals stained epidermal dendritic cells, while one monoclonal antibody was reactive with mast cells. The unique reactivity of 14 out of the 16 anti-DNA antibodies was also demonstrated in mouse skin (Table IV), further confirming the autoreactive nature of the observed reactivity with skin as well as the conservation of antigenic structures recognized by anti-DNA antibodies in different animal species.

In human SLE, several anti-nuclear staining patterns are well known [1]. Our seven anti-nuclear monoclonal antibodies from MRL-*1pr/1pr* mice were also classified into three different groups based on the nuclear staining pattern, i.e., diffuse, granular or peripheral, as noted with SLE sera (Table IV).

Two monoclonals (16-D-6, 3-B-4) showed positive nuclear staining with frozen sections of skin tissues despite their negative FANA staining with acetone-fixed HeLa S3 cells. Such a discrepancy may reflect the tissue difference or the difference in the preparation of substrate tissues for immunofluorescence.

Table IV. Reactivities of Anti-DNA Monoclonal Antibodies Against Human and Murine Skin

Monoclonal antibodies	Immunohistologic findings in human and murine skin
Group A	
1-C-1	Epidermal cells and some dermal cells (cytoplasmic)
1-C-B	No positive staining
1-G-6	Anti-nuclear antibody (diffuse)
2-E-11	Anti-nuclear antibody (diffuse)
3-H-12	Anti-nuclear antibody (granular)
7-C-6	Anti-nuclear antibody (diffuse) and anti-basement membrane
7-E-6	Epidermal cells (cytoplasmic)
14-C-8	Epidermal cells (cytoplasmic) in human skin, no positive staining in mouse skin
16-D-6	Anti-nuclear antibody (granular) in human skin, possibly mast cells in mouse skin
16-G-2	Anti-nuclear antibody (granular)
20-A-8	Suprabasal cells, some dendritic cells, and dermal cells
Group B	
3-A-8	No positive staining
3-B-4	Anti-nuclear antibody (peripheral)
4-E-12	Epidermal cells (cytoplasmic)
8-D-5	Mast cells
10-A-8	No positive staining
12-C-8	Epidermal basal cells, epidermal dendritic cells and dermal cells
13-C-11	No positive staining
15-B-12	Epidermal cells (cytoplasmic)
16-H-1	Suprabasal epidermal cells, some dendritic cells, and dermal cells

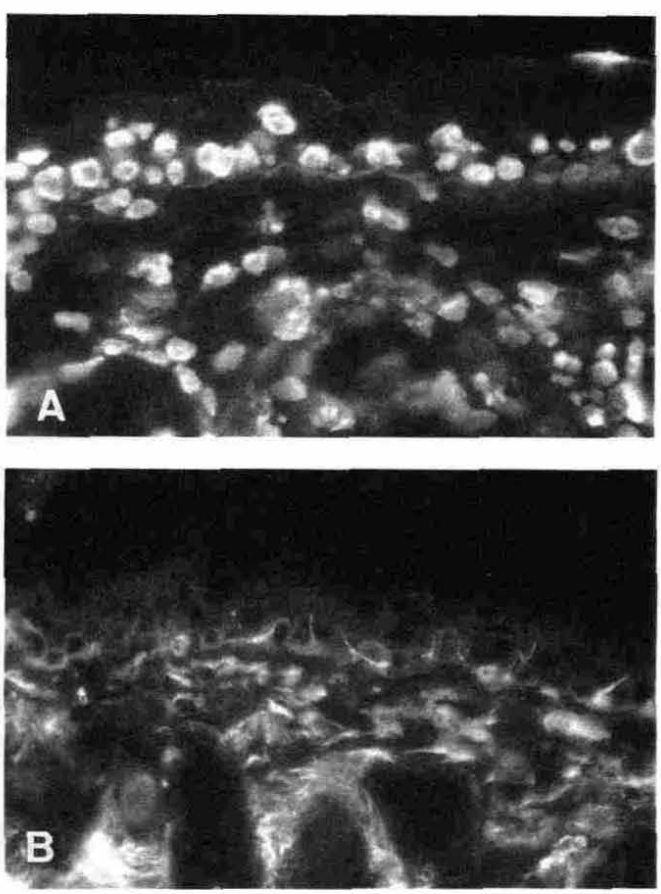

Figure 3. Immunofluorescence staining of normal mouse skin with anti-DNA antibodies. 16-G-2 stained nuclei and epidermal basement membrane (*A*), while 12-C-8 stained epidermal cells and epidermal dendritic cells (*B*) (× 450).

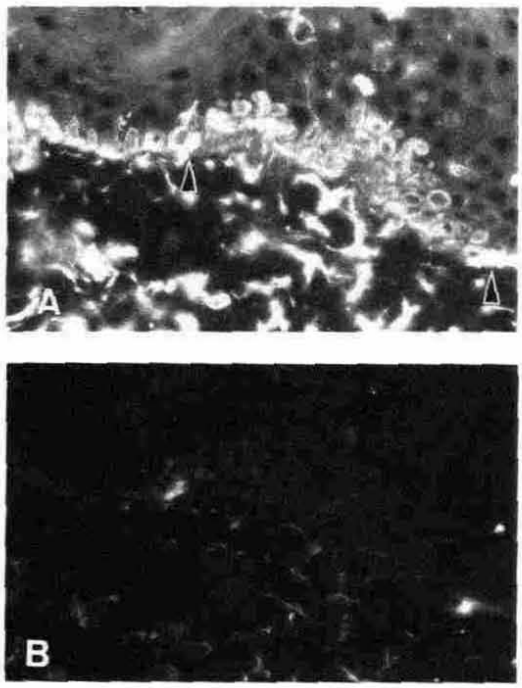

Figure 4. Double immunofluorescence staining of the same cryostat section of normal human skin with anti-DNA antibody and anti-tyrosinase antibody. 12-C-8-bearing cells are detected with FITC-labeled anti-mouse Ig (*A*), and tyrosinase-bearing cells are revealed using the combination of anti-tyrosinase antibody and rhodamine-labeled anti-rat Ig (*B*) (× 300).

One monoclonal anti-nuclear antibody, 7-C-6, was also found to stain the basement membrane zone. Faaber et al [8] have reported similar cross-reactivity of anti-DNA antibody with heparan sulfate. Heparan sulfate proteoglycan is one of the macromolecules found in the epidermal basement membrane [11], and our 7-C-6 was confirmed to react with heparan sulfate glycosaminoglycan in ELISA (data not shown). In human SLE, immunoglobulin deposits are detected in the epidermal basement membrane zone of lesional as well as affected skin. The latter phenomenon, designated as lupus band test, is considered to have a close correlation with the disease activity [21]. Furukawa et al [22] found that MRL-*lpr/lpr* mice also show a positive deposition of immunoglobulins at the dermo-epidermal junction. The same group of researchers [23] noted a significant age-related correlation between skin immunoglobulin deposition and serum anti-ssDNA antibodies, although they failed to demonstrate any DNA antigens at these locations. Thus, the present results strongly suggest that such anti-DNA antibodies with various cross-reactivities are present at such locations. Vacuolar degeneration of the epidermal basal cells is considered to be one of the hallmarks of histopathologic changes in the cutaneous lesions of LE [4]. Moreover, Sontheimer et al [24] reported that Langerhans cells become less dendritic and decrease in number, showing irregular distribution in cutaneous lupus erythematosus. The vacuolar degeneration of epidermal basal cells also constitutes characteristic changes in the skin of MRL-*lpr/lpr* mice [22]. In the present study, we found that one antibody, 12-C-8, was reactive with epidermal basal cells and Langerhans cells, the most severely damaged cells in LE skin lesions. These findings suggest that antibodies whose reactivity is similar to that of 12-C-8 might be involved in pathologic skin changes in human as well as murine lupus.

The demonstration of three monoclonal antibodies (12-C-8, 20-A-8, 16-H-1) that were reactive with melanocytes might also suggest the involvement of such antibodies in melanocyte damage noted in the skin lesions of SLE, particularly to that in the prominent depigmentation noted in old discoid lupus lesions. Moreover, 12-C-8 also stained Thy-1$^+$ dendritic epidermal cells as well as Langerhans cells in the murine epidermis. Thy-1$^+$ dendritic epidermal cells, whose function remains unclear, have been found in mouse skin [25,26], but not in rat or human skin [27]. Therefore, this antibody stains all kinds of dendritic cells noted in the epidermis.

In our studies five monoclonal antibodies were reactive with the cytoplasm of epidermal cells as well as some dermal cells. One monoclonal antibody (8-D-5) reacted with mast cell granules. However, at present, it is unclear whether such antibodies might be associated with any of the pathologic cutaneous changes in LE.

Possible mechanisms for the cell damage mediated by the cross-reactive antibodies cannot be discerned from this work. However, at least three mechanisms can be postulated, i.e. antibody-mediated lysis, complement-mediated lysis through antibody, and antibody-dependent cell mediated cytotoxicity (ADCC). Among them, Norris et al [28] clearly demonstrated that monocyte- or lymphocyte-mediated ADCC is by far the most effective against keratinocyte targets, whereas antibody alone or antibody plus complement produces only minimal lysis. Thus, if the skin changes of SLE as well as those of MRL-*lpr/lpr* mice were caused by autoantibodies with specificity similar to that of 12-C-8, the antibody-dependent cellular cytotoxicity seems to be most important.

As mentioned before, although certain anti-dsDNA antibodies have been associated with certain types of tissue damage in SLE, it has been a matter of debate whether anti-DNA antibodies are also involved in pathogenesis of cutaneous lesions of SLE. For example, by using adult human skin grafted onto nude mice, Lee et al [13] did not observe any binding of anti-dsDNA antibodies even in the skin exposed to ultraviolet light, while they found definite binding of anti-SS-A antibodies from patients with subacute cutaneous LE to human keratinocytes. Moreover, because the monoclonal antibodies employed in this study were produced from the spleen cells obtained from autoimmune MRL-*lpr/lpr* mice, it has yet to be determined whether anti-DNA antibodies with similar reactivities are also demonstrable in human SLE. At present, our data only show that most of the cross-reactive antigens in skin tissue are common to

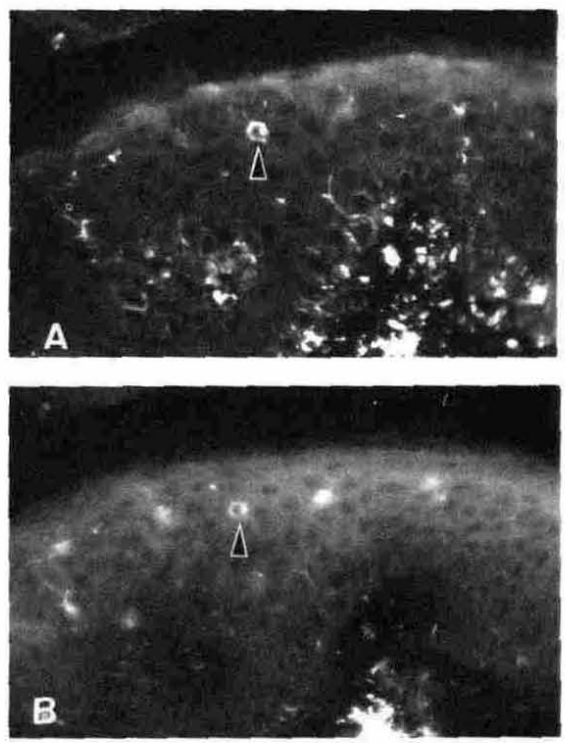

Figure 5. Double immunofluorescence staining of epidermal dendritic cells with anti-DNA antibody and with anti-HLA-DR antibody in the same cryostat section of normal human skin. 12-C-8-bearing cells are detected with FITC-labeled anti-mouse Ig (*A*) and HLA-DR-bearing cells are recognized by phycoerythrin-labeled anti-HLA-DR antibody (*B*) (× 300).

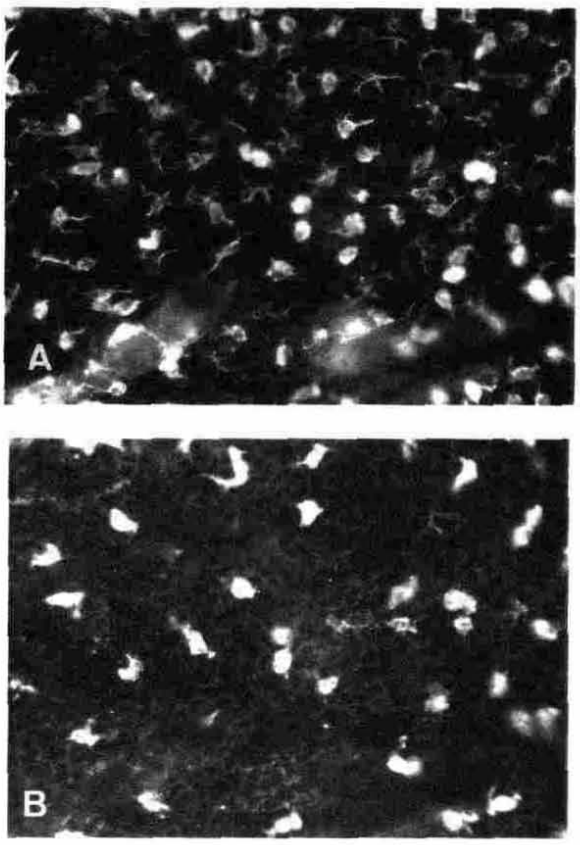

Figure 6. Double immunofluorescence staining with anti-DNA antibody and anti-Thy-1.2 antibody in the murine epidermal sheets. 12-C-8-bearing cells are recognized by FITC-labeled anti-mouse Ig (*A*), and Thy-1.2-bearing cells are detected by rhodamine-labeled anti-rat Ig (*B*) (× 300).

both humans and mice. However, it is plausible that such anti-DNA antibodies, if also produced in patients with SLE, might play a role in the pathogenesis of the LE skin lesions through the cross-reactive property.

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